A New Way to Find Protein-Protein Interactions

SEARCHING IN A NEW LOCATION

- **Cytoplasmic interactions allow posttranslational processing of target and bait proteins**
- **Two-hybrid screening with transcriptional activators and inhibitors**
- **Inducible system allows use and discovery of toxic proteins**

The CytoTrap™ two-hybrid system† is a new way to identify protein-protein interactions: searching in the cytoplasm instead of the nucleus of the yeast cell.[1] This innovative system is based on reestablishment of the Ras pathway to detect in vivo protein-protein interactions, expanding the utility of two-hybrid screening. This new system greatly increases the opportunities to find unique interactions, even if a conventional two-hybrid screen was unsuccessful. Conventional GAL4 and LexA systems rely on transcriptional activation of reporter genes in the nucleus to detect interactions. Unfortunately, proteins expressed in the nucleus do not have the opportunity for posttranslational modifications that might be critical for certain interactions. Furthermore, this approach eliminates the use of transcriptional activators or inhibitors as interaction partners. These restrictions have greatly limited the study of signal transduction pathways and many other pertinent cellular interactions that are now possible with the CytoTrap system.

CytoTrap™
Two-Hybrid System

STRATAGENE
An Agilent Technologies Company

The CytoTrap™ Two-Hybrid System

New Cloning Tools

Research projects have changed considerably in the last few years from cloning and sequencing new genes to determining the encoding protein's role in the living cell. One effective way to define gene function is to identify proteins that interact with your protein of interest. Protein-protein interactions are central to many cellular processes such as DNA replication, transcription, translation, splicing, secretion, cell cycle control, signal transduction and intermediary metabolism. Discovering these interactions will let you begin to elucidate the in vivo function of newly discovered genes and further develop an understanding of a known protein's function.

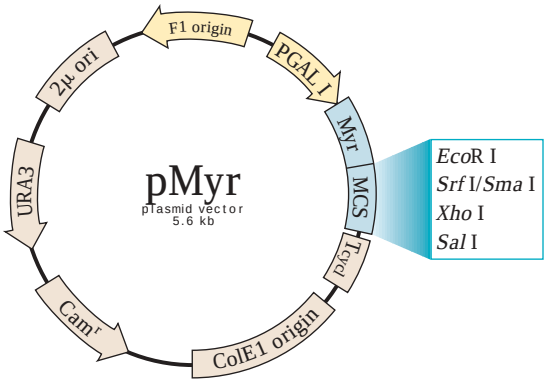
The first yeast two-hybrid system, developed by Dr. Stanley Fields and colleagues[2], allows researchers to screen a cDNA library to discover whether their protein of interest interacts with any other protein in the library. The Fields two-hybrid system and the second-generation versions that are currently used[2-4] exploit the modular nature of the DNA binding and the activation domains of a transcriptional activator. These two-hybrid systems find interactions between a gene of interest (bait) fused to the DNA binding domain and the cDNA library (targets) fused to the activation domain. In a positive interaction, the bait and target proteins reconstitute a transcriptional activator molecule in the nucleus of the cell that now activates transcription of a reporter gene in the yeast cell. However, these systems fail to detect proteins that intrinsically modulate transcription, (i.e. transcriptional activators or repressors), proteins that require posttranslational modification in the endoplasmic reticulum or in the cytoplasm, and proteins that require the environment of the cytoplasm or the cell membrane for interaction.

Ras Rescue

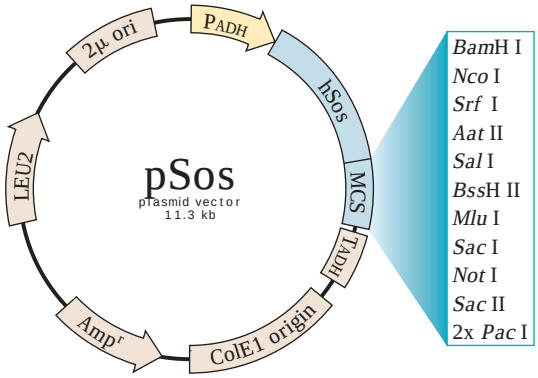
The CytoTrap two-hybrid system is a completely different way to discover protein-protein interactions. All interactions occur in the cytoplasm of the cell, so many new areas of investigation are now possible. Protein-protein interactions are detected by recruitment of the human Sos gene product (hSos) to the membrane of the cell, which activates the Ras pathway. The CytoTrap system contains the unique yeast strain cdc25H, which harbors a temperature-sensitive mutation in the cdc25 gene, the yeast homologue for hSos. This protein, a guanyl nucleotide exchange factor, is essential for activation of the Ras pathway and ultimately survival and growth of the cell. The mutation in the cdc25 protein is temperature sensitive; the cells can grow at 25˚C, but not at 37˚C unless rescued with a protein-protein interaction. This cdc25 gene can be complemented by the hSos gene product to allow growth at 37˚C, providing that the hSos protein is localized to the membrane via a protein-protein interaction.

Unique Vectors

The ability to rescue the cdc25H strain with the hSos protein provides the foundation for a novel two-hybrid system. The pMyr and pSos vectors complete the structure of this system. The pMyr vector is designed for cDNA library construction. Genes are expressed in this vector as a fusion protein with the *src* myristylation signal that targets and anchors the protein to the cell membrane with the gene product extruding into the cytoplasm. The bait protein is expressed as a fusion protein with the hSos protein from the pSos vector. When the cDNA library and the bait construct are cotransformed into the cdc25H yeast strain, the only cells that can grow at 37˚C are those that have been rescued by a protein-protein interaction recruiting hSos to the cell membrane.



- Myristylation signal to direct and anchor protein in membrane
- Ura3 gene allows selection in yeast
- Chloramphenicol resistance for selection in *E. coli*
- 2µ origin for high copy replication in yeast



- Human Sos gene for Ras rescue
- Leu2 gene for selection in yeast
- Ampicillin resistance for selection in *E. coli*
- 2µ origin for high copy replication in yeast

Demonstrated Success

To demonstrate the unique abilities of the CytoTrap system, we selected an interaction that could not be tested in a conventional two-hybrid system, the interaction of the transcription factor MafB with itself. This gene contains an endogenous nuclear localization signal, so we could also verify the pMyr and pSos vectors' ability to properly direct fusion proteins to the membrane and cytoplasm. MafB belongs to a large family of bZIP transcription factors that are expressed in a wide variety of tissues. MafB is reported to form homodimers with itself or form heterodimers with Fos and ATFS (but not with c-Jun) through the leucine zipper motif[5]. We created constructs in the pMyr and pSos vectors with the MafB gene inserted into each vector. As a negative control, we inserted c- Jun into the pMyr vector.

These constructs were introduced into the cdc25H yeast strain, selected at 25˚C, then transferred to fresh plates and grown at 37˚C for selection of positive interactions. As shown below, the MafB protein interacts with itself and with the viral form of c-Maf, but not with c-Jun. In addition, the pSos-MafB construct cannot rescue the cdc25H cells with an empty pMyr vector. Other known interaction partners were tested in the system, including the lambda *cI* protein that interacts with itself, the CD40 and TRAF-2 interaction, the interaction between the PI3 kinase catalytic subunit p110 and the PI3 kinase regulatory subunit p85 and the interaction between the Jun zipper domain and the Jun dimerization partner. All of these interactions were detectable as expected, proving the effectiveness of the CytoTrap system in detecting protein-protein interactions.

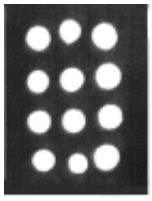
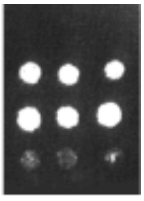
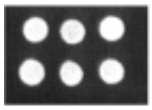
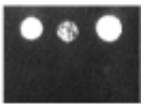
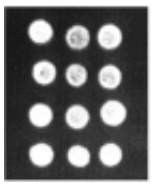
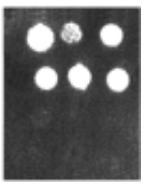
Complete Convenience

The CytoTrap two-hybrid system is available in a wide array of configurations for the flexibility to meet the needs of your next research project. The fastest way to begin is to go to our website and examine the premade library list. We might have already made a library for you. Each premade library meets the high standards that you expect from Stratagene including just one amplification. The cdc25H yeast strain, all control plasmids, the pSos bait vector and a comprehensive manual are also included to ensure your success. A complete library construction kit for directional cloning using oligo(dT) priming is also available. cDNA synthesis reagents, predigested pMyr vector, competent *E. coli* cells, yeast strain, pSos bait vector, all control plasmids and a complete manual are all included in the CytoTrap XR library construction kit. A kit with supercoiled plasmids is also available and individual plasmids can be purchased as well.

New Locations

The CytoTrap two-hybrid system gives you the best opportunity to find the proteins that your gene of interest is interacting with, whether it is a transcriptional activator, inhibitor, or just needs posttranslational processing.

Detection of Protein-Protein Interactions by the SOS System

pSos	pMyr	25˚C	37˚C
Sos	Myr		
CD40	TRAF-2		
p110	p85		
JZ	JDP-1		
cI	*cI*		
cI	Myr		
MafB	MafB		
MafB	v-Maf		
MafB	c-Jun		
MafB	Myr		

The SOS interaction trap system was used to detect various protein-protein interactions. A series of hSos fusion proteins which contain CD40 receptor, P13 kinase catalytic subunit p110, Jun zipper domain (JZ), Bacteriophage lambda repressor *cI* and transcription factor MafB; and a series of Myr fusion proteins which contain TRAF-2 (TNFR-associated factor), P13 kinase regulatory subunit p85, JDP-1 (Jun dimerization partner), *cI*, MafB, v-Maf (viral form of c-Maf), and c-Jun were constructed and tested.

Ras Rescue

1. Construct cDNA library in pMyr vector

2. Subclone bait protein of interest into pSos vector

3. Cotransform library and bait vector into cdc25H yeast strain

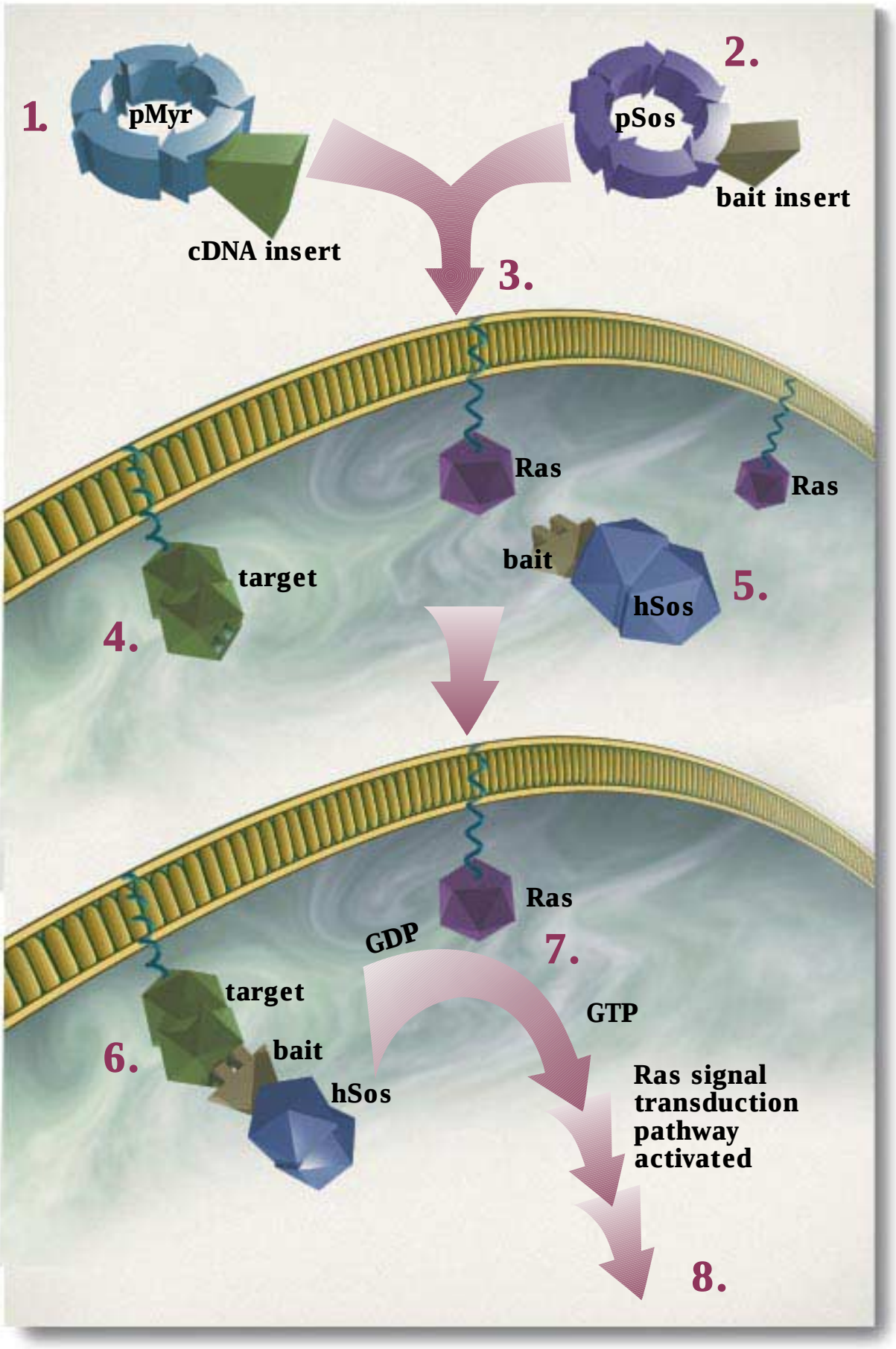
4. Target protein in pMyr is directed and anchored to cell membrane

5. Bait protein is expressed as fusion protein with human Sos

6. If target and bait proteins interact then hSos is brought into close proximity to Ras

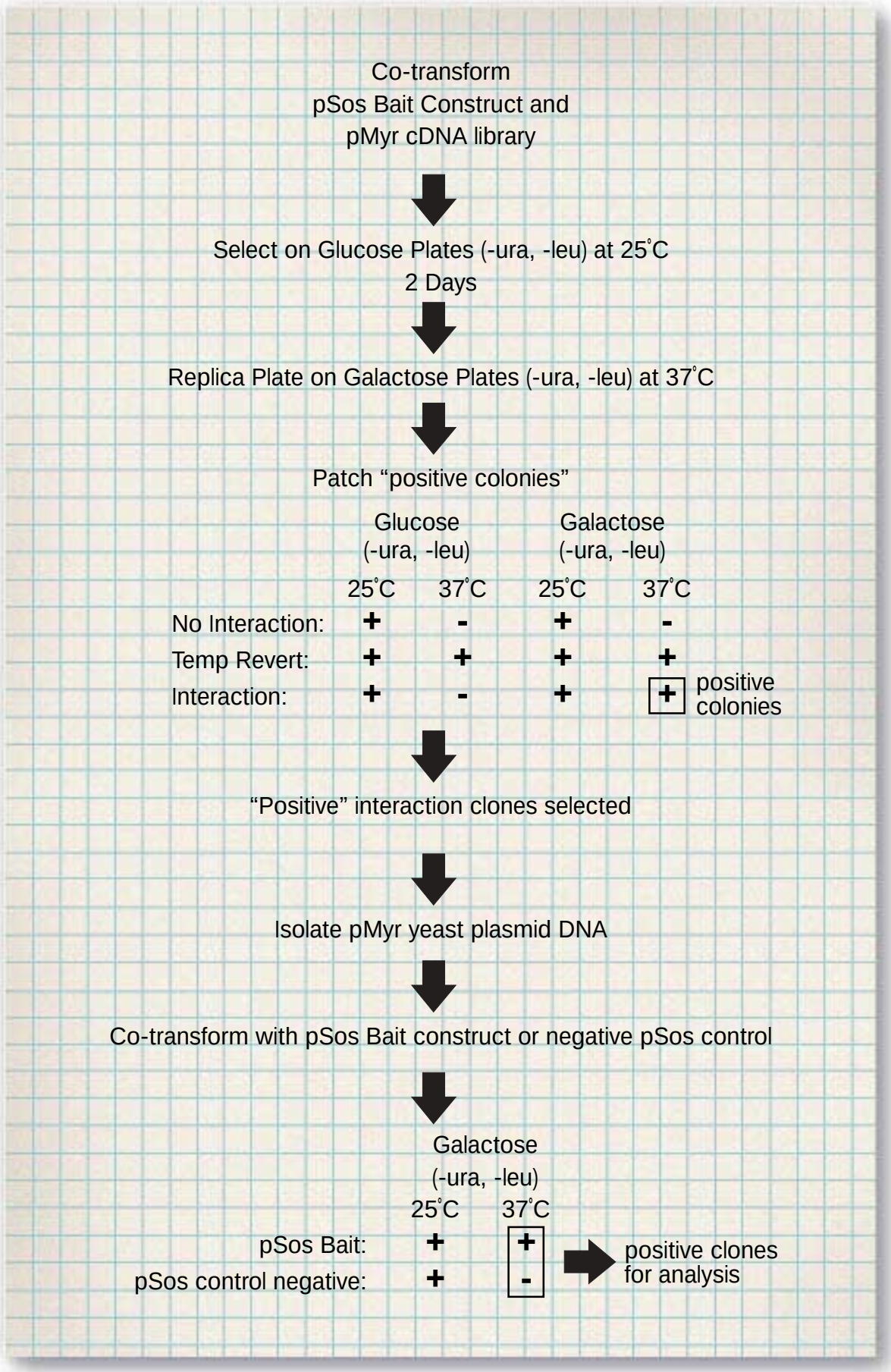
7. hSOS protein activates Ras by GDP/GTP exchange

8. Yeast survive and are selected by growth at 37˚C



CytoTrap™ Procedure

After a library is constructed in the pMyr vector and the bait is cloned into the pSos vector, the library can be screened. The GAL1 promoter in the pMyr vector is repressed when glucose is used as a carbon source and quickly induced in the presence of galactose, allowing expression of potentially toxic clones. After 2 days of growth under nonselective conditions, the screening plates are replica plated onto galactose medium and selected for protein-protein interactions at 37°C. Clones from colonies that exhibit galactose-dependent growth at 37°C are verified for a true interaction by showing bait-dependent growth on galactose medium at 37°C.



Ordering information

Visit the Stratagene website for a current list of premade libraries.

CytoTrap™ XR Library Construction Kit		
5 cDNA synthesis reactions	contents: cDNA synthesis kit, 5 µg pMyr XR predigested vector, pSos bait vector, 4 different control plasmids, 1.0 ml XL10-Gold® Kan Ultracompetent cells, and cdc25H yeast strain	#200444
CytoTrap™ Vector Kit		
Supercoiled vectors	contents: pSos bait vector, pMyr vector, 4 different controls (20 µg each), and cdc25H yeast strain	#217438
pMyr XR vector, predigested with *Xho* I and *Eco*R I (5 µg)		#217430
pMyr vector, supercoiled (20 µg)		#217431
pSos vector, supercoiled (20 µg)		#217433
cdc25H yeast strain		#217437

† NOTICE TO PURCHASERS:
U.S. Patent No. 5,776,689 entitled "Protein Recruitment System" and covering this two-hybrid system is owned by The Regents of the University of California and Baylor College of Medicine. Use of the two-hybrid system by commercial entities will require a license from The Regents of the University California. For license information, please contact:

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Printed in USA, April 2008
5989-8278EN